

**REMARKS**

Reconsideration is requested.

Claims 1-13 and 21-24 are pending. Claims 14-20 have been canceled, without prejudice.

The attached Request for Continued Examination includes a Petition for Suspension of Action for 3 months so that the applicants can arrange an interview with the Examiner, and the Examiner's Supervisor, to discuss the claims and what further may be required for allowance. The Examiner is requested to contact the undersigned to arrange an interview convenient to the Examiner's schedule in the event the Examiner reaches the case for Action prior to the undersigned contacting the Examiner to arrange an Interview. A separate Request for an interview is attached.

The objection to the Abstract is believed to be obviated by the above amendments and the attached new Abstract. Consideration of the attached and withdrawal of the objection to the Abstract are requested.

The objection to claim 13 is obviated by the above amendments, which incorporate the Examiner's helpful suggestion. Withdrawal of the objection is requested.

The Section 112, second paragraph, rejection of claims 4 and 5 is believed to be obviated by the above amendments. Withdrawal of the Section 112, second paragraph, rejection is requested.

The Section 112, first paragraph, written description" rejection of claims 10-11 is obviated by the above amendments. The claims have been revised to incorporate the N-terminal modifications and sequences of the proteins of SEQ ID NO:2, 4, 6 and 8,

which were in the applicants possession at the time of filing. Withdrawal of the Section 112, first paragraph "written description", rejection of claims 10 and 11 is requested.

The Section 112, first paragraph "written description", rejection of claim 13 is obviated by the above amendments. The revised claims refer to the proteins of SEQ ID NO:2, 4, 6 and 8, crystals of which were in the applicants possession at the time of filing the application. Withdrawal of the Section 112, first paragraph "written description", rejection of claim 13 is requested.

The Section 112, first paragraph "enablement", rejection of claims 10-11 is obviated by the above amendments. The applicants submit that one of ordinary skill in the art will be able to make and use the claimed invention, without undue experimentation, from the teachings of the present specification and the generally advanced level of skill in the art. Withdrawal of the Section 112, first paragraph "enablement", rejection of claims 10 and 11 is requested.

The Section 112, first paragraph "enablement", rejection of claim 13 is obviated by the above amendments. The applicants submit that one of ordinary skill in the art will be able to make and use the claimed invention, without undue experimentation, from the teachings of the present specification and the generally advanced level of skill in the art. Withdrawal of the Section 112, first paragraph "enablement", rejection of claim 13 is requested.

The Section 102 rejection of claims 1-4, 6-8, 10 and 21-23 over Kempf (1995, Archives of Biochemistry and Biophysics, vol. 321, p 277-288) is traversed. Reconsideration and withdrawal of the rejection are requested in view of the above and the following further comments.

The applicants submit, with due respect, that the rejection is based on a number of errors in analysis of the cited art and the applicant's previous response.

In regard to the Hepes buffer of Kempf, the Examiner is understood to imply that the applicants are relying on the Sigma catalog as evidence of the form of the compound used by Kempf. This is not the case. The Sigma catalog is submitted as evidence that both the acidic and salt form were commercially available and that a person of ordinary skill in the art would have been aware of this at the time. The evidence that Kempf used the acid form comes from the footnote on page 278 of Kempf, i.e., that the abbreviation "Hepes" is to N-[2-hydroxyethyl]-N'-[2ethanesulfonic acid]. There is no evidence that this definition is in error or that a person of ordinary skill in the art would have any reason to interpret this as defining the salt.

Under 35 USC 102, a "claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). There is no express nor inherent evidence that Kempf is mistaken in his description of N-[2-hydroxyethyl]-N'-[2ethanesulfonic acid]. Therefore the Kempf buffer AD with 150 mM NaCl as cited by the Examiner does not meet the requirements of a buffer having a salt concentration from 200 to 1000 mM.

Moreover, on page 279, right hand column, Kempf describes purification of [His]<sub>6</sub>-2D6-Δ25 in the presence of detergent. Kempf resuspends a cell pellet in a buffer comprising 150 mM NaCl. This buffer is outside the scope of the present claims, for the reasons set out above. The cells are lysed in the buffer and spun at a low speed to pellet debris. The supernatant is recovered and spun at high speed (142,000 g) to

pellet the membrane fraction. The supernatant and pellet are separated, the membrane fraction is resuspended in buffer AD (500 mM) and centrifuged again at high speed. The supernatant from both high speed centrifugations are combined, adjusted to 500 mM NaCl and detergent added. This mixture is then used to recover the [His]<sub>6</sub>-2D6- $\Delta$ 25.

Claim 1 requires that cells are recovered from culture in salt buffer having a salt concentration of from 200 to 1000 mM. Claim 1 further requires that the cells are lysed in this buffer.

The Examiner is understood to believe that the open-ended terminology of claim 1 means that Kempf can be interpreted to meet all the requirements of claim 1. Revised claim 1 specifies that the buffer in which the cells are suspended ("a first salt buffer") is the buffer in which the cells are lysed ("lysing cells in said first salt buffer").

Kempf teaches resuspending cells and lysing said cells in a buffer of 150 mM salt. The use of 500 mM salt (buffer AD) cited by the examiner occurs only after, and not before cell lysis. Accordingly, claim 1 is not anticipated by Kempf.

Since Kempf fails to describe all the elements of claim 1, the remaining claims, which all incorporate the requirements of claim 1 are also novel over the cited art.

Withdrawal of the Section 102 rejection is requested.

The Section 103 rejection of claim 5 over Kempf (1995, Archives of Biochemistry and Biophysics, vol. 321, p. 277-288) in view of Anderson (1968, Journal of Bacteriology, vol. 96, p 93-97) is traversed. Reconsideration and withdrawal of the rejection are requested in view of the above and the following further comments.

The Examiner is understood to have suggested the applicants have argued that Kempf use 21% of detergent. The applicants respectfully submit that the Examiner has misstated or misunderstood the applicants previous comments. Applicants noted on page 19 of the previous response that:

“In particular, as can be seen from Table 1(B) on page 282 of Kempf et al, the recovery of P450 2D6 in the presence of detergent was 21%.” [emphasis added here]

In other words, the process used by Kempf results in a loss of about 4/5 (i.e. 79%) of the [His]<sub>6</sub>-2D6-Δ25 present at the start of the process. The applicants' point, which has been misconstrued or misunderstood by the Examiner, is that the present invention provides for recovery of about 4/5 of the P450 present at the start of the process - see Tables 1 and 2 in Example 1(a) of the instant specification. Thus the present process is on the basis of this comparison about four times more efficient than that of Kempf which evidences that the two processes are not the same.

The Examiner is also understood to have suggested that the instant claims as written do not “exclude any additional method steps as long as those steps includes the step recited in the instant claims in order in view of open-ended term “comprising” and the use of alphabet labeling each method step”. See sentence spanning pages 14-15 of the Office Action dated September 6, 2007. In response, applicants submit that since the present claims recite the lysis of cells in a salt buffer having a salt concentration of from 200 to 1000 mM it is not possible, regardless of the open-ended language of the claims, to lyse cells more than once, i.e. in both the salt buffer of instant claim 1 and then again in a buffer according to Kempf.

On page 15 of the Office Action, the Examiner states that:

“for one skilled in the art, it has been known that high salt in the buffer would dissociate the membrane associating proteins (specially periplasmic membrane proteins) from the membrane part by general salting in or out process. One skilled in the art knows the overall purification steps are designed to balance between the factors comprising final yield and the purity according to characteristics of protein such as the stability. As long as the protein is stable, having additional separation step such as removing cytosolic part from the cell lysate would increase the purity of membrane associated protein since a purity of protein is considered as one of important factors in order to use it for a crystallization of protein, although not necessarily, because impure proteins have been crystallized in the past.”

The applicants submit, with due respect, that these statements of asserted fact are unsupported by reference to any document and provide no substantiated reasons as to why, starting from Kempf, a person of ordinary skill in the art would have been motivated to perform the process of the instant claims.

The Examiner has acknowledged the applicants submissions that Kempf seems to have “perceived need in the art to resuspend host cells expressing P450s in a buffer with low ionic strength”. The present invention departs from this perception. The Examiner has failed to provide any indication of any motivation or teaching in Kempf for departing from the process set out in the citation. The Examiner has also failed to point out any teaching or motivation from Anderson as to why a person of ordinary skill would have modified Kempf to arrive at the method of instant claim 1.

Moreover, as noted above, the process of the instant invention is demonstrated to provide a 4-fold (i.e. 400%) improvement in recovery of P450 over that taught by Kempf. There is no indication in either Kempf or Anderson that improvements on this scale could be made, nor has the Examiner provided any reasons as to how or why a

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person of ordinary skill would have departed from the teachings of the cited art to arrive at the instant claims, in the expectation of an improvement of this magnitude.

Withdrawal of the Section 103 rejection is requested.

The Examiner is requested to hold in abeyance the obviousness-type double patenting rejection of claims 1-12 and 21-23 over claims 1-12 of copending application Serial No. 10/221,036 until such time as allowable subject matter is identified.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested. The Examiner is requested to contact the undersigned in the event anything further is required.

An interview with the Examiner and the Examiner's Supervisor is requested prior to the Examiner's issuance of a further Action on the merits. A Petition for suspension of Action is being filed herewith to ensure an interview may be arranged prior to the Examiner's next Action.

Respectfully submitted,

**NIXON & VANDERHYE P.C.**

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